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14. ABSTRACT

The goals of this section are to characterize the physiological, histological, cerebral vascular and behavioral effects of blast-induced neurotrauma (BINT) and to determine the effects of hemorrhagic hypotension (HH) after mTBI on physiological and pathophysiological outcome. These studies indicated that BINT resulted in reduced CBF and increased CVR, observations consistent with the presence of cerebral vasospasm. Our results that BINT impaired vasodilatory responses to reduced intravascular pressure in middle cerebral arterial segments, suggest that blast injury may increase vulnerability of the brain to the effects of hemorrhagic hypotension. These results are consistent with our observations that rats subjected to mild fluid percussion TBI prior to severe HH exhibit higher numbers of injured hippocampal neurons than rats subjected to HH without mTBI.

15. SUBJECT TERMS
blast injury; fluid percussion injury; traumatic brain injury; hemorrhagic hypotension

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Table of Contents

Page

Section I: Introduction

Basic research designed to improve the diagnosis and outcome after MTBI requires the use of reproducible animal models that replicate important patho-physiological features of TBI in patients. Any of the widely used experimental TBI models can be modified to cause mild injury, but typically the clinical features of MTBI have not been assessed. In addition, there are few well-characterized models of blast-induced neurotrauma (BINT) that isolate the effects of blast to the brain rather than the body. The goal of this section of the project is to thoroughly characterize the physiological, histological, cerebral vascular and behavioral effects of BINT and to determine the effects of hemorrhagic hypotension after mild fluid percussion TBI on physiological and pathophysiological outcome.

mTBI Followed by Hemorrhagic Hypotension

Although the incidences of hypotension, hypoxemia and other posttraumatic injuries and the statistical correlations between posttraumatic injuries and mortality vary to some extent among patient studies, in general, clinical and experimental evidence both support a strong association between posttraumatic insults and increased mortality and morbidity after TBI (DeWitt & Prough, 2003; 2009). In one of the first reports of the effects of posttraumatic hypotensive or hypoxemic insults after TBI, Miller & Becker (1982) observed that hypotension (systolic blood pressure < 90 mmHg) or hypoxemia ($PaO_2 \leq 60$ mmHg) doubled mortality rates and markedly reduced the percentage of good recovery/moderate disability patients. Chesnut et al. (1993) confirmed these observations in a larger series of patients and also observed that, while combined hypoxemic and hypotensive insults were associated with worsened outcome than either insult alone, hypotension alone was a more important predicator of outcome than hypoxemia alone. In a retrospective analysis of data from more than 1,200 TBI patients, Luerssen and Klauber (1989) observed that reductions in mean arterial blood pressure of as little as 10 mmHg were associated with a two-fold increase in mortality in TBI patients. While these and other clinical and experimental observations indicate that the injured brain is especially vulnerable to the effects of secondary insults such as hemorrhagic hypotension (HH), virtually all previous research has investigated the consequences of secondary insults after moderate to severe TBI. There is very little research on the consequences of posttraumatic hypotension after mild traumatic brain injury (mTBI). Therefore, we are conducting experiments designed to establish the effects of mTBI followed by HH on cerebral blood flow (CBF), cerebral vascular reactivity and histopathological and behavioral outcome.

Blast-induced neurotrauma (BINT)

Blast injury is the most common cause of mortality and morbidity in combatants in Operations Iraqi Freedom and Enduring Freedom and BINT is one of the most common causes of mortality. Even mild BIBI may be associated with chronic cognitive and emotional deficits. The development of effective therapies for BIBI requires experimental models that replicate important features of BINT in humans. Currently, there are few well-characterized models of BINT that isolate the effects of blast to the brain rather than the body. We developed a new experimental model of explosive BINT based on .22 and .27 caliber blank cartridges. The goal of this project is to thoroughly characterize the physiological, histological and behavioral effects of mild fluid percussion injury, controlled cortical impact and BINT.

SA #1. **To standardize animal models of MTBI utilizing clinically important endpoints.**

A large percent of civilians and military personnel with TBI suffer from a trauma that can be classified as mild. Subtle damage that occurs in MTBI patients may or may not be evident by CT scans or neurological examinations in the acute phase (hours) after injury. However, in the chronic phase (weeks to months) many of these patients exhibit profound neurobehavioral deficits that can hinder their ability to hold a job or maintain normal interpersonal relationships. These deficits include working memory impairments, episodic memory deficits, impaired cognitive speed, depression, heightened stress/anxiety, sleep disorder and PTSD-like symptoms. Unfortunately, the paucity of reproducible animal models of blast injury has limited research on the pathophysiology of blast injury and many important features have not been investigated. Therefore, the goal of following studies has been to develop and thoroughly characterize a new rodent model of BINT.

Section II: Progress to Date:

mTBI Followed by Hemorrhagic Hypotension

During the previous period of support, we continued measurements of arterial blood pressure and perfusion in rats subjected mTBI followed by severe hemorrhagic hypotension and resuscitation (HH). Adult, male Sprague-Dawley rats were anesthetized with 1.5% isoflurane, intubated and mechanically ventilated on a mixture of 70:30 air:oxygen, placed in a stereotaxic headholder and prepared for mild fluid percussion TBI and for measurements of cerebral perfusion using lasers Doppler flowmetry (LDF). Briefly, the left calveria lateral and slightly posterior to the injury adapter was thinned and a fiberoptic needle probe in a stereotaxic electrode holder was placed over the shaved parietal calvaria and carefully positioned away from large vessels visible in the remaining calvaria. Measurements of cerebral perfusion were recorded on a PeriFlux PF3 Laser Doppler Perfusion Monitor (Perimed, Stockholm, Sweden) and compared between rats as a percentage change from baseline. Rats were then randomly assigned to receive sham injury/sham HH, mTBI alone, HH alone or mTBI followed by HH. In the groups receiving HH, blood was withdrawn to reduce mean arterial blood pressure (MAP) to approximately 40 mmHg. Half of the blood was withdrawn within 5 minutes, then an additional 25% over the next 5 minutes and the final 25% over the next 5 minutes. After an additional 30 min of HH, the "prehospital care" resuscitation phase was instituted by infusing saline (0.9% NaCl) in 1 ml boluses to achieve a MAP of at least 50 mmHg for 30 minutes. This was followed by a "definitive hospital care" phase for 30 minutes. During this

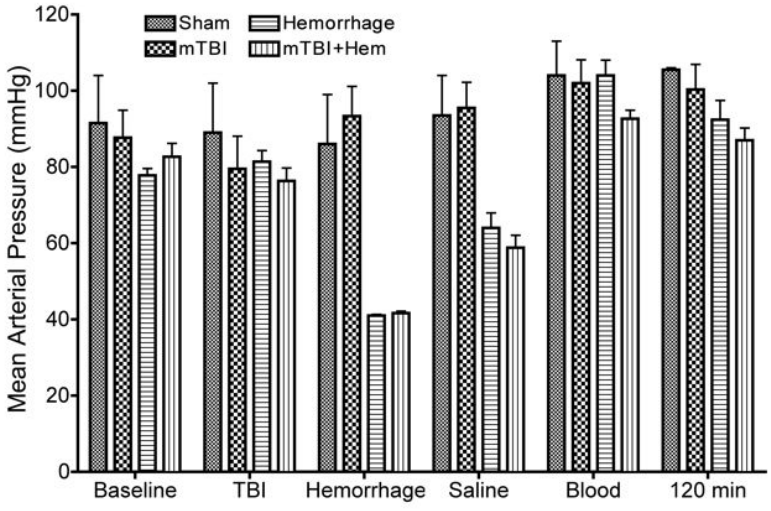

Figure 1 – Mean arterial blood pressure in rats sham injury (Sham, n=2), mTBI alone (mTBI, n=6), hemorrhagic hypotension (HH) followed by resuscitation alone (Hemorrhage, n=5) or mTBI followed by HH (mTBI+Hem, n=6).

time, the rats were ventilated on 100% oxygen, and the shed blood was reinfused. Cerebral perfusion was recorded at baseline immediately prior to injury, immediately following mTBI, at the end of the hemorrhage, at the end of the saline "prehospital care" resuscitation, at the end of the "definitive hospital care" shed blood reinfusion and 120 min after TBI.

Blood pressure decreased in the groups subjected to HH during the hemorrhage phase then returned to baseline levels following resuscitation (Figure 1). In the Sham and mTBI groups, blood pressure remained constant throughout the experimental period.

Cerebral perfusion decreased during hemorrhage in the mTBI and mTBI + HH groups (Figure 2). Cerebral perfusion returned to baseline during saline and blood resuscitation in all groups. These results indicate that cerebral perfusion was reduced during the hemorrhage period in both groups that received mTBI. Despite the lack of hemorrhage, cerebral perfusion was reduced after TBI in the mTBI only group. The most pronounced reductions in cerebral perfusion were observed in the mTBI + HH group. These observations suggest that even mTBI is associated with reduced cerebral perfusion and that hemorrhage after mTBI is associated with further reductions in perfusion.

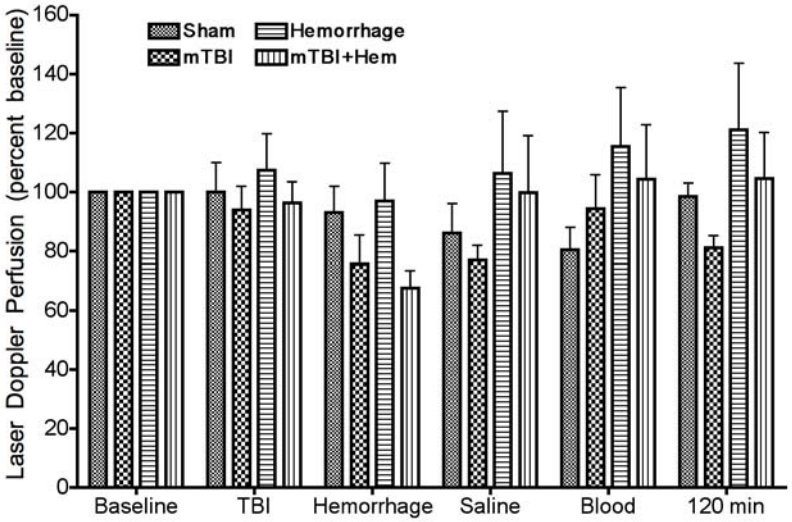

Figure 2 – Laser Doppler measurements of cerebral perfusion in rats sham injury (Sham, n=2), mTBI alone (mTBI, n=6), hemorrhagic hypotension (HH) followed by resuscitation alone (Hemorrhage, n=5) or mTBI followed by HH (mTBI+Hem, n=6).

Blast-induced neurotrauma (BINT) – Advanced Blast Simulator

In order to compare the effects of the combined blast exposure plus blunt impact injury produced by the Vandenberg blast device with those of pure blast injury, we acquired an Advanced Blast Simulator designed by David Ritzel and manufactured by Steve Parks at ORA, Inc. (Fredericksburg, MD). As noted in our previous quarterly report, the ABS was delivered, installed and tested by David Ritzel and Steve Parks. In order to accurately record the extremely rapid blast waves, the pressure transducers must collect readings at the rate of 50,000 – 100,000 samples per second. With five sensors, each blast produces pressure data at the rate of 250,000 – 500,000 samples per second. To permit the analog-to-digital conversion and high rates of data collection required, we purchased an upgrade to our current laboratory data acquisition system (BioPac) that has the necessary sampling capacity. During the previous period of support, we received and programmed the BioPac and we've begun our studies of pure blast exposure.

The ABS has blast pressure sensors located on top of the specimen chamber in front of, directly over and behind the animal positioning tray (Figure 3, pressure sensors 1, 2 and 3, respectively). A fourth pressure sensor is located on the side of the chamber very close to the

animal positioning tray (Figure 3, pressure sensor 4). A fifth pressure sensor is located in the driver chamber (not shown on Figure 3).

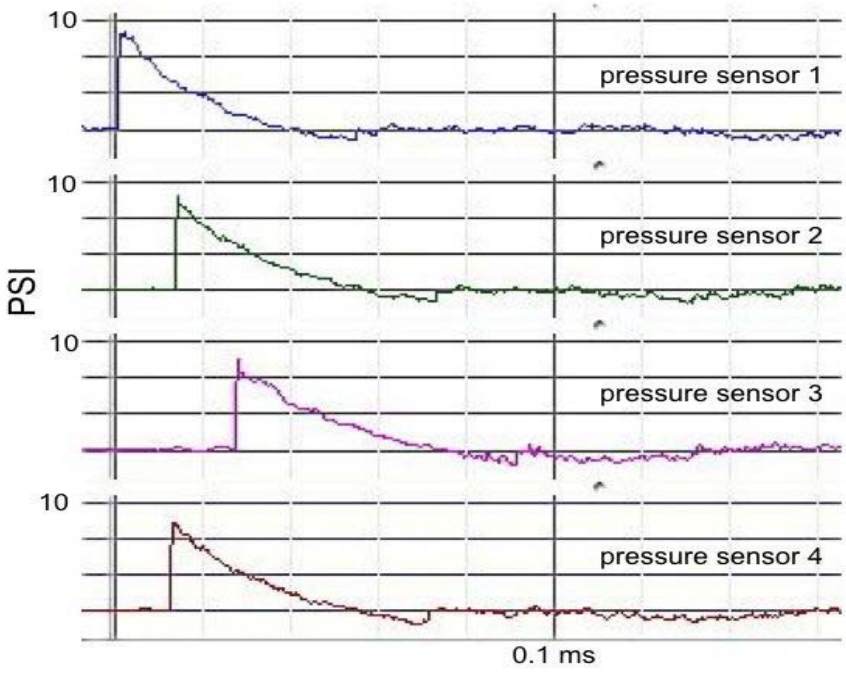

Figure 3 – Blast pressure traces recorded from the Advanced Blast Simulator (ABS). Pressure sensors 1, 2 and 3 are located on the top wall (ceiling) of the specimen chamber upstream, level with and downstream of the rat positioning tray, respectively. Pressure sensor 4 is located in the side wall of the specimen chamber immediately adjacent to rat positioning tray.

The ABS produced Friedlander-type blast overpressures and underpressures. Peak pressures were highest in sensor 1, closest to the blast driver chamber and lowest in sensor 3, farthest from the blast. Blast waves in sensors 2 and 4 were identical, consistent with their locations equidistant from the driver chamber.

These studies demonstrate that the ABS produces ideal blast waves characterized by an extremely rapid rate of rise and a slower decay followed by a brief period of underpressure.

Dilator Responses to Reduced Intravascular Pressure in Middle Cerebral Arteries (MCAs) Harvested from Rats Subjected to ABS Blast Injury

To determine the effects of primary blast injury on cerebral vascular responses to reduced intravascular pressure, adult, male, Sprague-Dawley rats (n = 7) were anesthetized with isoflurane (4.0%), intubated and secured to the ABS animal tray and the animal tray was positioned so that only the head of the animal was within the ABS specimen chamber. On return of a withdrawal response to paw pinch, rats were subjected to approximately 18 psi of peak static shock wave pressure. Immediately, the rats were removed from the animal tray and righting reflex testing was performed. The time required for each rat to right itself from a supine position three times was recorded

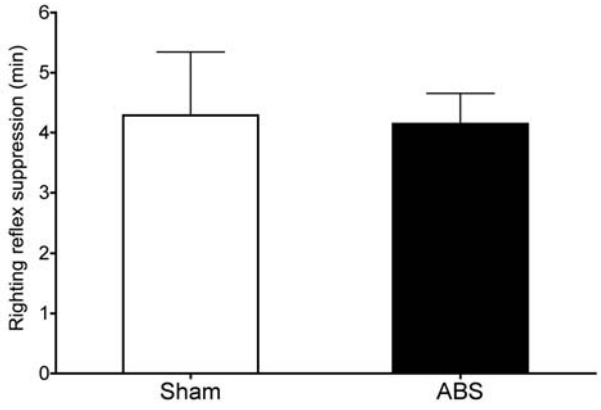

Figure 4 – Duration of righting reflex suppression in rats subjected to Advanced Blast Simulator (ABS) injury (n = 7) or Sham injury (n = 4).

and isoflurane (2.0%) anesthesia was resumed. Thirty minutes later, the isoflurane was increased to 4.0%, rats were decapitated and the MCAs were removed and mounted on glass micropipettes for measurements of arterial diameter changes in response to reduced intravascular pressure as described (Mathew, et al., 1999; DeWitt, et al., 2001).

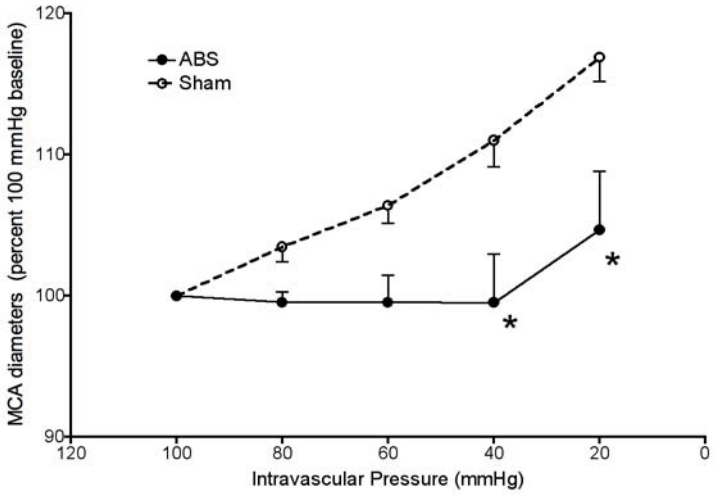

Figure 5 – Middle cerebral arterial (MCA) diameters during progressive reductions in intravascular pressure. MCAs harvested from rats subjected to ABS injury (18 psi, n = 7) or Sham injury (n = 4).

Rats subjected to 18 psi ABS injury exhibited durations of righting reflex suppression equal to those recorded in sham-injured rats (Figure 4). Despite the absence of an effect of blast exposure on duration of righting reflex suppression, responses to reduced intravascular pressure in MCAs harvested from rats subjected to 18 psi ABS blast were reduced significantly ($P < 0.05$, ABS vs. Sham)(Figure 5).

These results indicate that ABS blast pressure levels too low to produce behavioral changes (i.e. righting reflex suppression) were associated with significant impairment of cerebral vasodilatory responses to reduced pressure. These results suggest that primary blast exposure produced impaired responses to reduced intravascular pressure and, therefore, cerebral autoregulatory responses to reduced blood pressure without significantly altering consciousness.

Blast-induced neurotrauma (BINT) – Vandenberg Model of Primary Blast Combined with Blunt Impact TBI: Behavioral effects of blast injury followed by blunt impact injury

Although we initially believed that the Vandenberg device produced a pure, primary blast injury, subsequent study of high speed videos and discussions with experts in blast physics (David Ritzel, personal communication) and blast injury (Iboja Cernak, personal communication) convinced us that the model produces a blast wave followed by a jet flow of escaping gases. The concentrated column of gases and particles produces an impact that rapidly accelerates the head. Since the animals are placed on a thick pad of "memory foam" the rapid acceleration is followed by a rapid but slower deceleration. Therefore, we now consider the Vandenberg model to be one of primary blast exposure followed by blunt impact injury.

To determine the effects of combined blast injury followed by blunt impact, adult, male, Sprague-Dawley rats were anesthetized with isoflurane (4.0% in an anesthetic chamber) and placed beneath the Vandenberg device. Their heads were shaved and covered with a silicone pad that transmits the blast wave but protects the rats from flash burns. Rats were randomly assigned to receive sham injury (no blast, n = 4), mild blast injury (n = 4), mild-moderate blast injury (n = 5) or moderate-severe blast injury (n = 5). After injury or sham injury, motor function

(neuroscore), vestibulomotor function (beam balance & beam walking tasks) and working memory (Morris water maze) were assessed daily for three days.

The neuroscore is a series of motor function tests (McIntosh, et al., 1989):

1. hindpaw grasp – hindpaw grasp of a finger touching hindpaw = 0 (normal); no grasp = 1
2. forelimb flexion – forelimb extension when approaching a surface = 0 (normal); forelimb flexion = 1
3. hindlimb flexion – hindlimb extension when approaching a surface = 0 (normal); hindlimb flexion = 1
4. visual placement – forelimb extension when approaching a table edge = 0 (normal); no extension or forelimb flexion = 1
5. contact placement – forelimb extension when whiskers brush a table edge = 0 (normal); no extension or forelimb flexion = 1

The beam balance (Feeney, et al., 1982) and beam walking (Dixon, et al., 1999) tasks are widely used assessments of vestibulomotor function. For the beam balance task, rats are placed on a 1.5 cm wooden beam and scored on their ability to remain on the beam using a five-point scale. Rats remaining on the beam for the testing period (60 seconds) receive a score of 1 while those that fall off immediately receive a 5. For the beam walking task, rats are trained to traverse a wooden beam (165 x 2 cm) with three steel pegs equidistantly spaced and a darkened goal box at the far end. Once trained, rats are timed during three consecutive trials with time to goal box as the primary endpoint.

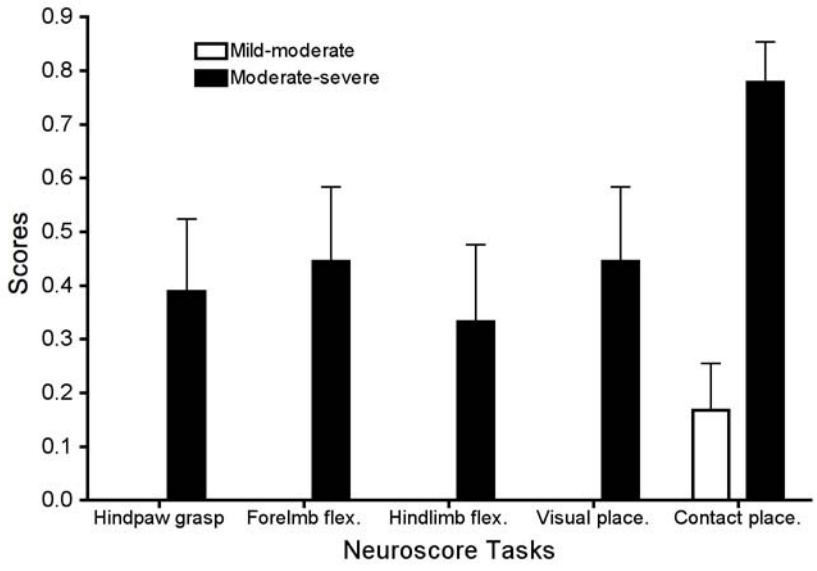

Figure 6 – Neuroscores in rats subjected to sham or mild, mild-moderate or moderate-severe Vandenberg blast injury.

Rats subjected to sham blast or mild blast injury received "normal" neuroscores of zero for all tasks (Figure 6). After mild-moderate blast injury, rats scored normally for all tasks except visual placement in which they demonstrated significant impairment. After moderate-severe blast injury, rats exhibited significant impairment in all tasks compared to sham rats and rats subjected to mild or mild-moderate blast injury.

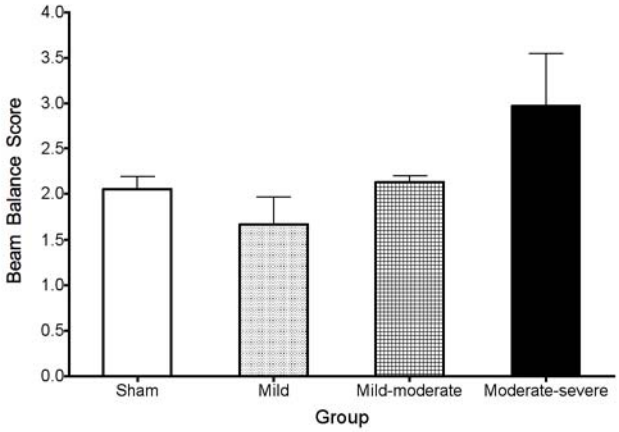

Figure 7 – Beam balance scores in rats subjected to sham, mild, mild-moderate or moderate-severe Vandenberg blast injury.

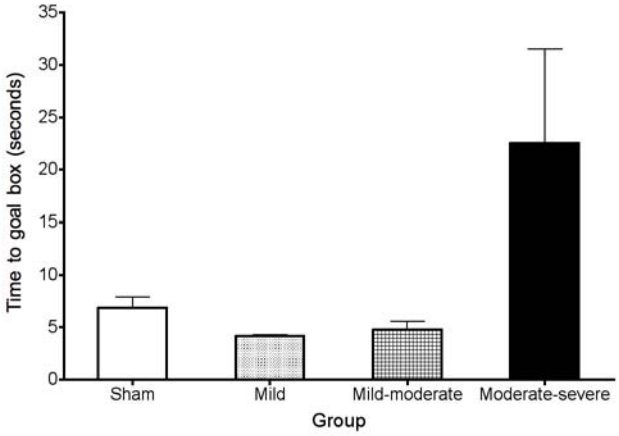

Figure 8 – Beam walking times in rats subjected to sham, mild, mild-moderate or moderate-severe Vandenberg blast injury.

There were no significant differences in beam balance scores (Figure 7) or beam walking times to the goal box (Figure 8) after Vandenberg blast injury.

Working memory was tested on post-blast days one through three using the Morris water maze. Rats were placed in one of four quadrant, allowed to find a hidden platform and then remain on the platform for 30 seconds. The rats were immediately placed back in the tank from the same starting position and the time required to find the platform was recorded. This procedure was repeated three times and the latencies to find the platform the second time were averaged

For this preliminary analysis, MWM latencies from all blast animals were averaged and compared to latencies from sham injured rats (Figure 9). On days one and two, latencies in the rats subjected to Vandenberg blast injury were longer than those in sham rats. On post-blast day three, the latencies in the two groups were nearly identical. These results suggest that working memory was impaired by blast injury but improved over time. Further studies with more rats per group are required to determine whether working memory is impaired by blast injury.

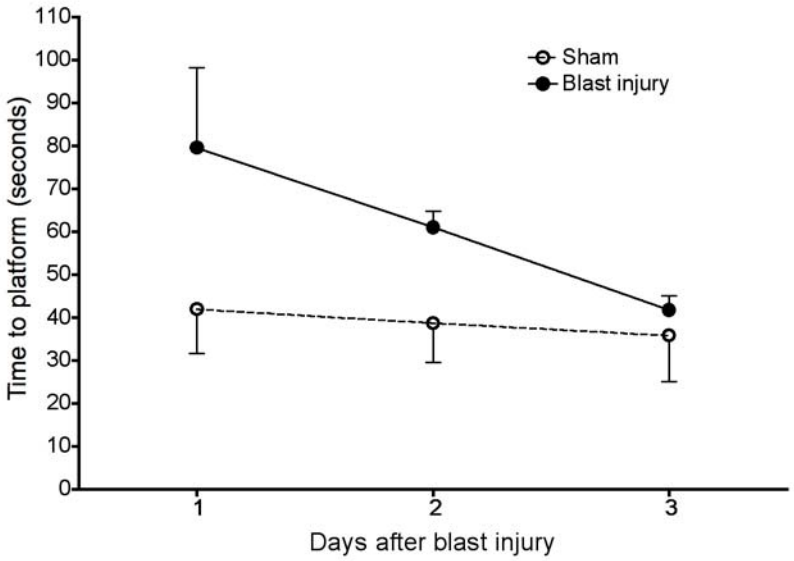

Figure 9 – Morris water maze latencies in rats subjected to sham injury or Vandenberg blast injury.

Key Research Accomplishments

- Mild fluid percussion TBI is associated with reduced cerebral perfusion.
- Hemorrhage after mTBI is associated with further reductions in perfusion.
- Preliminary studies using the Advanced Blast Simulator indicate that mild, primary blast injury significant reduced dilator responses to reduced intravascular pressure in the absence of suppression of the righting reflex. *These novel and important results suggest that primary blast injury, at levels too low to alter consciousness, may impair the capacity of the cerebral vasculature to respond to reductions in arterial blood pressure.*
- As the result of conversations with Dr. Ibolja Cernak and Mr. David Ritzel, internationally recognized experts on the pathophysiology and physics of blast injury, respectively, *we have concluded that the Vandenberg model is a novel, clinically relevant model of combined primary blast exposure followed by blunt impact injury.*
- Moderate-severe Vandenberg blast injury results in neurological (neuroscore), vestibulomotor (beam balance/beam walking) and memory deficits (MWM).
- Mild-moderate Vandenberg injury is associated with deficits in a specific visual placing task.

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Reportable Outcomes

Manuscripts:

We have submitted the following manuscript for consideration for publication in a special issue of the *Journal of Neurotrauma* on mTBI. We received reviewers' comments and have revised and resubmitted the manuscript:

DeWitt, DS, Perez-Polo, R, Hulsebosch, C, Dash P, Robertson, C, Experimental Models of Mild Traumatic Brain Injury

The 2010 Galveston Brain Injury Conference focused on blast injury. The following summary of the meeting was published in the *Journal of Neurotrauma*:

Masel, BE, Bell, RS, Brossart, S, Grill, RJ, Hayes, RL, Levin, HS, Rasband, MN, Ritzel, DV, Wade, CE, DeWitt, DS, Galveston Brain Injury Conference 2010: Clinical and Experimental Aspects of Blast Injury, J. Neurotrauma 29(12):2143-2171, 2012

Presentations:

None

Abstracts:

The following abstracts were presented at the 2012 National Neurotrauma Symposium, 23 – 26 July, 2012:

Ruppert KA, Parsley MA, Boone DK, Prough DS, MD. DeWitt DS, PhD. Effects of Mild Blast-Induced Neurotrauma on Blood-Brain Barrier Permeability in a Complex Blast Rodent Model

Wynne KE, Zeng Y, Prough DS, DeWitt DS. Carboxyfullerene Nanoparticles Reduce Oxidative Stress after Rapid Stretch Injury In Vascular Smooth Muscle Cells, *in vitro*.

Perez-Polo JR, Rea H, Johnson K, Marsley M, Unabia G, Xu GY, DeWitt DS, Grill R, Hulsebosch C. Outcomes of mild traumatic brain injury in a rodent model. J. Neurotrauma 29:A63, 2012

Sell SL, Boone D, Prough DS, DeWitt DS, Hellmich HL. Estradiol effects on oxidative stress response genes in the cerebral vasculature after traumatic brain injury. J. Neurotrauma 29:A69, 2012

Zeng Y, DeWitt DS, Prough DS. L-NAME reduces levels of reactive oxygen species & improves gap junction coupling after rapid stretch injury in vascular smooth muscle cells, *in vitro*. J. Neurotrauma 29:A120, 2012

Conclusions

Mild fluid percussion TBI is associated with reduced cerebral perfusion and hemorrhagic hypotension after mTBI is associated with further reductions in perfusion. Preliminary studies using the Advanced Blast Simulator indicate that mild, primary blast injury significant reduced dilator responses to reduced intravascular pressure in the absence of suppression of the righting reflex. These novel and important results suggest that primary blast injury, at levels too low to alter consciousness, may impair the capacity of the cerebral vasculature to respond to reductions in arterial blood pressure. The Vandenberg model is a novel, clinically relevant model of combined primary blast exposure followed by blunt impact injury. Moderate-severe Vandenberg blast injury results in neurological (neuroscore), vestibulomotor (beam balance/beam walking) and memory deficits (MWM). Mild-moderate Vandenberg injury is associated with deficits in a specific visual placing task.

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Dixon CE, Kochanek PM, Yan HQ, Schiding JK, Griffith RG, Baum E, Marion DW, and DeKosky ST: One-year study of spatial memory performance, brain morphology, and cholinergic markers after moderate controlled cortical impact in rats. J Neurotrauma 16: 109-22, 1999

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